REMARKS

Prior to this response, claims 1-5 were pending. By the present communication, the chemical structure of (CBZ – arginine)$_2$ rhodamine 110 on page 42 of the Specification has been deleted and replaced with a corrected chemical structure. The originally filed chemical structure for this compound was inadvertently incorrectly drawn, as would immediately be recognized by those of skill in the art. This compound is a commercially available and well-known enzymatic substrate that is synthesized by coupling the carboxy group of the amino acid derivative to the amine groups of the rhodamine 110 dye. Thus, Applicants submit that correction of an error in the chemical structure of the substrate does not constitute new matter.

In the claims, claims 6 and 7 have been added and claims 1 and 3 have been amended to more clearly define the invention. The new claim language adds no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1-7 are currently pending.

Rejection under 35 U.S.C. § 112, First Paragraph – Written Description

Applicant respectfully traverses the rejection of claims 1-5 under 35 U.S.C. § 112, first paragraph, for containing subject matter that allegedly is not adequately described in the Specification so that those of skill in the art would understand that Applicant had possession of the claimed invention at the filing of the application. Specifically, the Examiner alleges that the Specification shows that the Applicant had described the invention in such a way that those of skill in the art would come to the conclusion that Applicant "had possession of the invention" only with respect to identifying *E. coli* clones comprising DNA isolated from a picoplankton sample wherein the clones express enzymes having hydrolase activity which are active after heating to 70° C for 45 minutes. The Examiner further alleges that the genome of the host cell would have to be known before those of skill in the art could determine whether the "desired characteristic" of enzymatic activity being produced was endogenously produced by the host cell or resulted from the "recovered DNA" placed into the host cell. In the Advisory Action, the

Examiner asserts that Applicant has failed to describe how the enzymatic activity produced by a "recovered DNA" could be distinguished from enzymatic activities produced endogenously by the host cell when the conditions are not at extremes of temperature or pH. Applicant respectfully disagrees with these conclusions.

Applicant has previously argued that given the knowledge of the art regarding enzyme activity tests (which the Examiner acknowledges is a large body), those of skill in the art could easily determine whether every clone in the library was giving the same positive signal or displaying the same enzymatic characteristic, or whether one or more particular cells was producing a positive response that was *not common to the whole of the library* and hence attributable to the "recovered DNA." Once that determination is made and the responsible host cells noted, those of skill in the art would know how to obtain and further screen the product of the recovered DNA. Thus, Applicant respectfully submits that a subtraction technique does not at all require use of a host cell whose complete genome is known, but can be accomplished simply by ignoring, screening out, or "subtracting out" as described in the Specification the enzymatic activities that are commonly produced by the host cells and this procedure can be followed at any condition of temperature and pH to allow discovery of enzymatic activities produced by clones in a library that are not produced by the host cells (i.e., are attributable to the "recovered DNA."

With regard to the subject matter of claim 5, Applicant again respectfully submits that those of skill in the art do not need to be told the exact temperature or pH at which the host's enzymes cease to fully function in order to practice the claimed invention. In a test for an enzyme that maintains stability of enzymatic activity at elevated or lowered pH or temperature, those of skill in the art would understand that the subtraction process consists simply of incrementally raising (or lowering) the pH or temperature of the expression products until enzymatic activity common to all of the host cells has been eliminated. Only specific expression products then remain (each attributable to the "recovered DNA" contained in a particular host cell) that maintain enzymatic activity at the extreme pH or temperature. Thus, in one aspect, the invention assay is designed to discover enzymes encoded by uncultivated organisms whose

activity is maintained in the face of the extreme condition to which the library products are submitted. Accordingly, false positives produced in common by the host cells are easily eliminated in a library setting in a type of "subtraction" procedure simply by increasing the extremity of the condition on the expression products beyond that at which the host cell's enzymes (which are common to all of the clones) are active, by analogy with Applicant's description in the Specification of heating *E. coli* to 70 °C to inactivate host enzymes.

Moreover, Applicant respectfully submits that the Examiner's comments in the Advisory Action in response to the above line of reasoning are flawed because the test for written description of the invention is not the unskilled experimenter, but those of skill in the art relevant to the invention at hand. Applicant submits that those of skill in the art of expression libraries using host cells very quickly become familiar with the ranges of pH and temperature tolerance of a host cell found useful for such expression libraries. In addition, those of skill in the art would know, for instance, the pH and temperature preference of a host cell simply by consulting the scientific literature or based on knowledge of the pH and temperature conditions of the host's natural environment. If such familiarity has not already been acquired, those of skill in the art would conduct a few routine and simple tests *on the host cell alone* to determine its ranges of pH and temperature tolerance using the knowledge of the literature concerning the natural environment of the host cell as a starting point. Thus, Applicant respectfully submits that the Examiner is applying to the present invention a "heightened" level of description that is inappropriate to the subject matter of the invention at hand under the written description requirement of Section 112, first paragraph.

Accordingly, in view of the above amendments and arguments, Applicant respectfully submits that those of skill in the art would understand that the invention as described and as presently claimed was fully contemplated at the filing of the application.

In addition, Applicant disagrees with the Examiner's assertion in the Advisory Action that the phrase "two or more" which replaced the phrase "at least one" in the amendment filed on September 22, 2003 constitutes new matter because the Specification repeatedly refers to "clones" in the plural form. However, to expedite prosecution and reduce the issues, Applicant

has further amended claims 1 and 3 to delete the term at issue and to refer, respectively, to "a mixture of clones" or "a mixed DNA population of uncultivated organisms" in the library. Support for the new claim language is found in the specification as follows: "The screening for chemical characteristics may be effected on individual expression clones or may be initially effected on *a mixture of* expression clones to ascertain whether or not the mixture has one or more specified enzyme activities" (Specification, page 21, lines 8-10; emphasis added). Thus, Applicant submits that the issue of new matter as to the phrase "two or more" is now moot.

In view of the above amendments and remarks, Applicant submits that claims 1-5 meet all requirements under 35 U.S.C. § 112, first paragraph, and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 112, First Paragraph – Enablement

Applicant respectfully traverses the rejection of claims 1-5 under 35 U.S.C. § 112, first paragraph as allegedly lacking an enabling disclosure for the full scope of the claims. Claims 3-5 have previously been amended to underscore that the subject matter of the claims and the type of "characteristic" being assayed is limited to enzymes and enzymatic activities.

Applicant's remarks above concerning the description of the invention pertain equally and are incorporated here with respect to enablement.

Particularly with respect to claims 4 and 5, it is believed that those of skill in the art would consider as "routine" the testing for enzymatic activity to discover enzymes having stability of enzymatic activity under extreme conditions, such as elevated or lowered pH or temperature. Thus, Applicant submits that no special skill is required to accomplish such screenings and the methods of the invention do not involve highly unpredictable subject matter. The test for "undue experimentation" is whether an unusual degree of skill or inventiveness is required, not whether the testing is repetitive and/or routine. Accordingly, Applicant again respectfully submits that claims 1-5 are fully enabled by the description of the Specification, and reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement are respectfully requested.

The Rejection under 35 U.S.C. § 103(a)

Applicant respectfully traverses the rejection of claims 1-5 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Yen et al. (U.S. Patent No. 5,171,684; hereinafter "Yen") in view of More et al. (Appl. Environ. Microbiol. 60(5):1572-1580, 1994; hereinafter "More").

The invention methods for identifying clones of a recombinant library produced from DNA derived from uncultivated organisms which express an enzyme with a desired characteristic, as defined by amended claim 1, distinguish over the combined disclosures of Yen and More at least by requiring: "screening in the liquid phase a library of a mixture of expression clones produced from DNA randomly selected from uncultivated organisms, said screening being effected on expression products of said clones to thereby identify clones which express an enzyme with a desired characteristic. The invention methods, as defined by amended claim 3, distinguish over the combined disclosures of the cited art by requiring:

(i) recovering DNA randomly selected from a mixed DNA population of uncultivated organisms by contacting the recovered DNA in a liquid phase assay under hybridizing conditions with at least one hybridizing probe containing a full-length coding region sequence or a partial coding region sequence for an enzyme having the specified enzymatic characteristic;

(ii) transforming a host cell with the recovered DNA to produce a library of clones, wherein the DNA is modified or mutagenized prior to forming the library of clones; and

(iii) screening for a specified enzymatic characteristic in an expression product prepared by expressing the library of clones to obtain expression products, which are screened to identify the specified enzymatic characteristic.

Applicant respectfully submits that Yen fails to suggest the invention methods of claims 1 and 3 because Yen fails to disclose any method for screening for enzymes obtained from a mixed population of uncultivated organisms whose DNA was randomly selected for formation of the library. By contrast, in Yen's method the isolated DNA was obtained from *a single cultured* organism whose enzymatic products were known, and the DNA was pretreated so as to bias the

DNA towards a particular known enzyme using a restriction endonuclease whose active site was known to exist in some or all of the genes encoding the predetermined target enzyme.

Thus, Applicant submits that Yen fails to suggest creating and screening a DNA library or a library of a mixture of clones that is produced from *DNA that is randomly selected from a mixture of uncultivated organisms* (as required by claims 1 and 3; emphasis added). In the Advisory Action, the Examiner has interpreted the claims as encompassing the situation wherein DNA is randomly distributed among the clones in a library and the Examiner observes that random distribution of the DNA of organisms among the clones of a library is always the case (Advisory Action, page 6).

However, to clarify Applicant's true conception of the invention, claims 1 and 3 have been amended to emphasize that the DNA to be put into the clones is "randomly selected" (i.e., no preselection process), not that the selected DNA is randomly distributed among the clones in the library as the Examiner has construed Applicant's claims. When this aspect of the invention is understood, it can be seen that the invention methods proceed in a manner that is exactly opposite to the method of library preparation followed by Yen, who obtains all of the DNA of a single cultivated organism and then goes through a series of steps designed to assure that the library includes only members that contain PmKR1 toluene monooxygenase genes (as described in the "Conjugation and Complementation and Screening Assay" of Example 3 of Yen). Moreover, there is nothing in Yen to suggest mutagenesis of *mixed DNA* for detection of a specified enzymatic characteristic that has increased pH or temperature stability, as is required by amended claim 3.

Thus, Yen not only fails to suggest the invention, but Applicant submits that Yen would fail to motivate those of skill in the art to "randomly select" the DNA recovered from a mixed population of organisms for formation of a library to be screened with an activity specific substrate because Yen actually "teaches away" from the crux of the invention, i.e., screening randomly selected mixed DNA from uncultivated organisms in a single library.

Moreover, Applicants submit that in view of the thrust of Yen's disclosure, those of skill in the art would not be led by Yen to look to More for further guidance. More details many

failures and problems encountered in isolating DNA from an environmental sample and More concludes that even if the extraction efficiency were improved to 99.9%, there would still be 10^6 cells per gram of sediment whose DNA *could not be accessed by current methods* (page 1578, Col. 2). Despite this, the Examiner asserts in the Advisory Action that More shows that isolation and purification of DNA from an SDS-treated sediment sample containing a mixed population of organisms is possible. However, Applicant submits that the Examiner's rosy picture of More's contribution fails to take into account the teachings of the reference as a whole as is required by the statute. For the reasons set forth in detail in the previous office action, Applicant submits that those of skill in the art would not have a reasonable expectation of success in accomplishing the invention methods based on the combined disclosures of Yen and More.

In particular Applicant submits that, even if motivated by More's analysis of the procedures for isolation and purification procedures to adapt Yen's type of assay along the lines of the invention methods, those of skill in the art would not have a reasonable expectation that Yen's disclosure regarding screening of a single gene derived from a single cultivated organism for enzyme activity could be adapted to an assay in which the *randomly selected* DNA of a mixed population of uncultivated organisms forms the library.

Accordingly, Applicant submits that *prima facie* obviousness of claims 1-5 is not established over the combined disclosures of Yen and More and reconsideration and withdrawal of the rejection are respectfully requested.

In view of the above amendments and remarks, Applicant respectfully submits that all claims are now in condition for allowance, which is respectfully requested. If the Examiner would like to discuss any issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Enclosed is Check No. 573629 in the amount of $760.00 ($250.00 for the Notice of Appeal fee, and $510.00 for the Three (3) Months Extension of Time fee). The Commissioner is hereby authorized to charge for any additional required fees, or credit any overpayments to Deposit Account No. 07-1896.

Respectfully submitted,

Reg. No. 45,517

Date: 1/26/2005

for

Lisa A. Haile, J.D., Ph.D.
Registration No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

DLA PIPER RUDNICK GRAY CARY US LLP
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133
USPTO CUSTOMER NO. 28213